Each morning we are born again. What we do today is what matters most-
Gautama Buddha

DNA REPLICATION

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General Features of DNA Replication

- The double-helical model for DNA includes the concept that the two strands are complementary. Thus, each strand can in principle serve as the template for making its own partner.
- This is called **semiconservative** replication because each daughter duplex has one parental strand and one new strand.
- Another potential mechanism is **conservative** replication, in which the two parental strands stay together and somehow produce another daughter helix with two completely new strands. Yet another possibility is random **dispersive** replication, in which the DNA becomes fragmented so that new and old DNAs coexist in the same strand after replication.
- In 1958, **Matthew Meselson and Franklin Stahl** performed an experiment to distinguish among these three possibilities.

The Meselson-Stahl experiment:

Escherichia coli grown in medium containing 15N, a heavy isotope of nitrogen. 15N contains one more neutron than the naturally occurring 14N. Unlike radioisotopes, 15N is stable and is not radioactive. After growing several generations of bacteria in the 15N medium, the DNA of E. coli became denser because the nitrogenous bases had incorporated the heavy isotope.

The density of the strands was determined using a technique known **as density-gradient centrifugation.**

A solution of cesium chloride (CsCl) – a heavy metal salt – containing the DNA samples is spun in an ultracentrifuge at high speed for several hours. Eventually, an equilibrium between centrifugal force and diffusion occurs, such that a gradient forms with a high concentration of CsCl at the bottom of the tube and a low concentration at the top. DNA forms a band in the tube at the point where its density is the same as that of the CsCl. The bands are detected by observing the tubes with ultraviolet light at a wavelength of 260 nm, in which DNA absorbs

(a)- Cells were grown for many generations in a medium containing only heavy nitrogen, 15N, so that all the nitrogen in their DNA was 15N, as shown by a single band (blue) when centrifuged in a CsCl density gradient.

(b)- Once the cells had been transferred to a medium containing only light nitrogen, 14N, cellular DNA isolated after one generation equilibrated at a higher position in the density gradient (purple band).

(c)- Continuation of replication for a second generation yielded two hybrid DNAs and two light DNAs (red), confirming semiconservative replication.

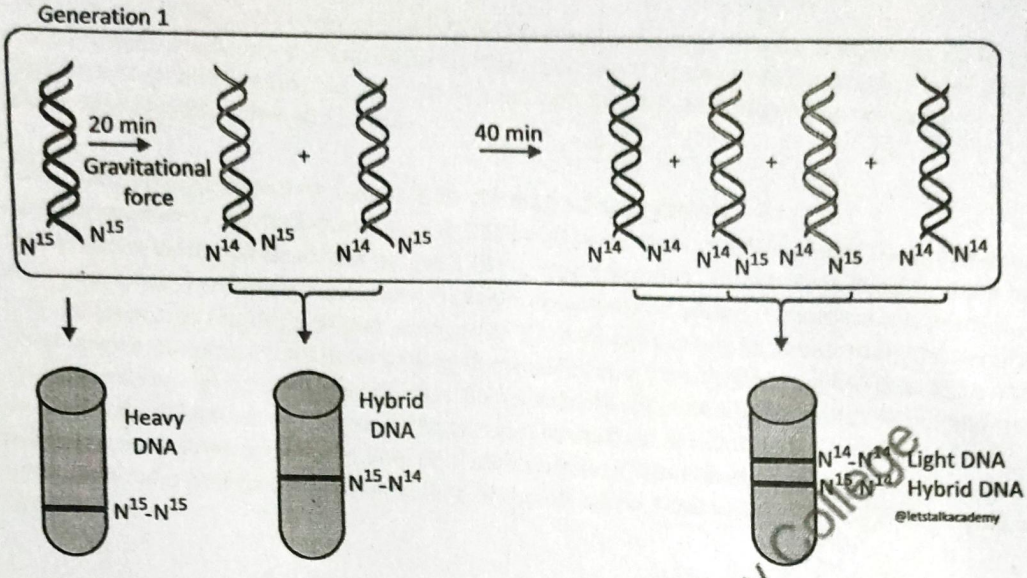
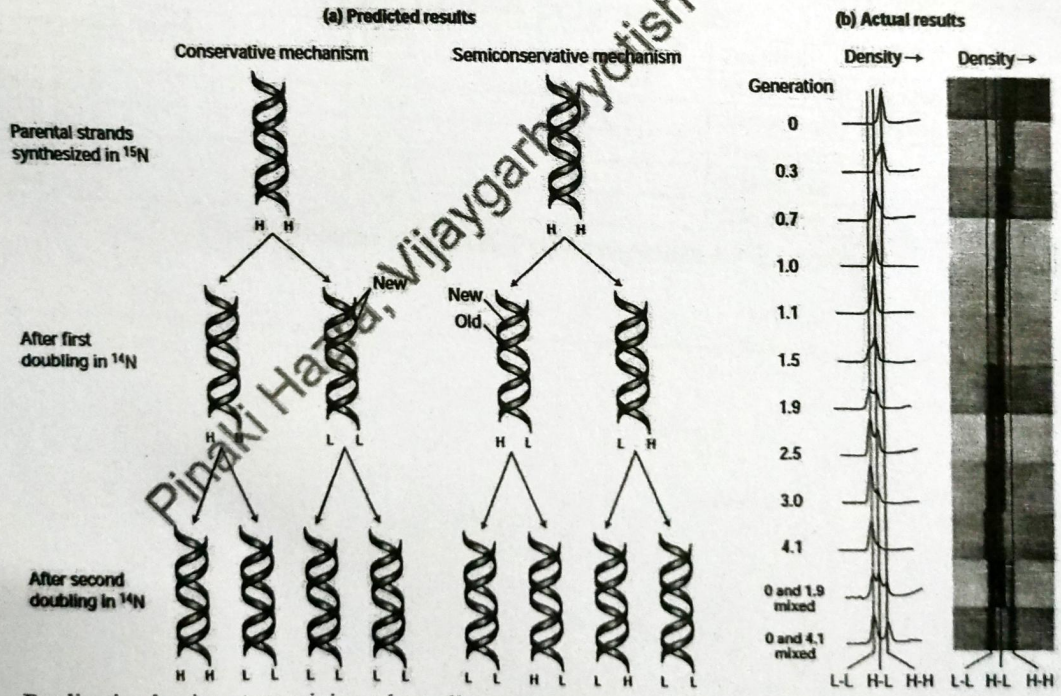
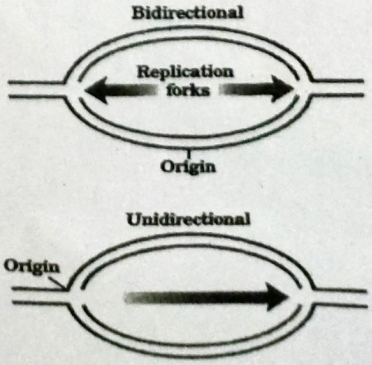


Fig. : The Meselson-Stahl experiment after replication



Replication begins at an origin and usually proceeds **bidirectionally**. Replication is a highly coordinated process in which the parent strands are simultaneously unwound and replicated. The evidence was provided by **John Cairns**, using autoradiography.

In this experiment, DNA was radiolabelled by using radiolabeled thiamine with tritium $_1H_3$.



Addition of $_1H_3$ for a short period just before the reaction is stopped allows a distinction to be made between unidirectional and bidirectional replication, by determining whether label (red) is found at one or both replication forks in autoradiogram. **This technique has revealed bidirectional replication in *E. coli*.**

DNA polymerases are template-directed enzymes.

DNA polymerases add nucleotides to the 3'OH end of a polynucleotide chain. The polymerase catalyzes the **nucleophilic attack** by the 3'OH -group terminus of the polynucleotide chain on the a phosphoryl group of the nucleoside triphosphate to be added .To initiate this reaction, DNA polymerases require a *primer* with a free 3'OH group already base-paired to the template. They cannot start from scratch by adding nucleotides to a free single-stranded DNA template. RNA polymerase, in contrast, can initiate RNA synthesis without a primer All DNA polymerases have structural features in common The three-dimensional structures of a number of DNA polymerase enzymes are known. The first such structure was elucidated by **Tom Steitz and coworkers**, who determined the structure of the so-called *Klenow fragment* of DNA polymerase I from *E. coli*.

Types of DNA polymerases:

Polymerase Name	Gene	Function
DNA Pol I	PolA	Major repair enzyme
DNA Pol II	Pol B	Minor repair enzyme
DNA Pol III	Pol C	True replicase
DNA Pol IV	Din B	SOS repair
DNA Pol V	D2C	SOS repair

Comparison of DNA Polymerases of E. coli:

DNA Polymerase I, Klenow Fragment

DNA Polymerase I, Klenow Fragment (Large Fragment) is a proteolytic product of *E. coli* DNA Polymerase I, which possesses the 5'→3' polymerase and 3'→5' exonuclease activities of intact DNA Polymerase I, but lacks the 5'→3' exonuclease activity of full-length DNA Polymerase I. The lack of 5'→3' exonuclease activity enables the DNA Polymerase I, Klenow Fragment to be used in random primer labeling and DNA sequencing experiments in addition to second-strand cDNA synthesis. The enzyme is supplied in a buffer of 50 mM potassium phosphate (pH 6.5), 1 mmDTT and 50% glycerol (2140A/B) or ethylene glycol (2140AK/BK).

Replication Fidelity:

ccurReplication Fidelity means _How aate the DNA replication?' In *E. coli*, a mistake is made only once for every 10^9 to 10^{10} nucleotides added. For the *E. coli* chromosome of $4.6\ 10^6$ bp, this means that an error occurs only once per 1,000 to 10,000 replications. The discrimination between correct and incorrect base pairing is based on:

- Hydrogen bonding behavior
- Geometry of standard base paring

One mechanism intrinsic to virtually all DNA polymerases is a separate 3'-5' exonuclease activity that double-checks each nucleotide after it is added. This nuclease activity permits the enzyme to remove a newly added nucleotide and is highly specific for mismatched base pairs. If the polymerase has added the wrong nucleotide, translocation of the enzyme to the position where the next nucleotide is to be added is inhibited. This kinetic pause provides the opportunity for a correction. The 3'-5' exonuclease activity removes the mispaired nucleotide, and the polymerase begins again. This activity is known as **proofreading**.

Nick translation:

In this process, an RNA or DNA strand paired to a DNA template is simultaneously degraded by the 5'-3' exonuclease activity of DNA polymerase I and replaced by the polymerase activity of the same enzyme. These activities have a role in both DNA repair and the removal of RNA primers during replication.

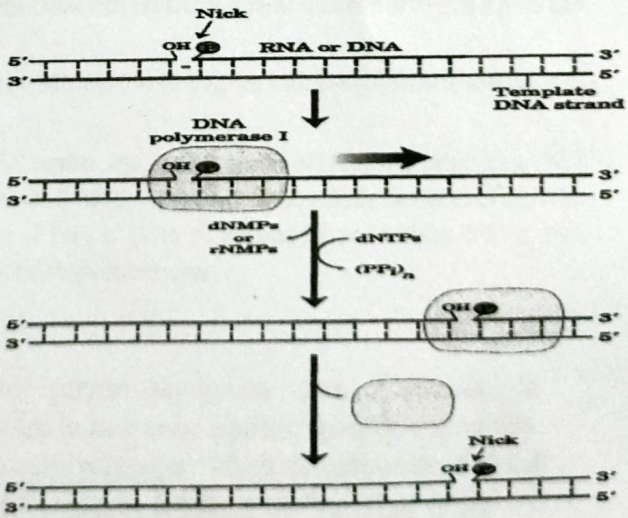


Fig: Nick translation

The strand of nucleic acid to be removed (either DNA or RNA) is shown in green, the replacement strand in red. DNA synthesis begins at a nick (a broken phosphodiester bond, leaving a free 3' hydroxyl and a free 5' phosphate). Polymerase I extends the nontemplate DNA strand and moves the nick along the DNA—a process called nick translation. A nick remains where DNA polymerase I dissociates, and is later sealed by another enzyme.

Enzymes of DNA replication:

DNA polymerase

DNA polymerase catalyzes the polymerization of deoxynucleotide triphosphate (dNTP) and synthesizing a new DNA strand on a template strand. DNA polymerase adds new nucleotide at 3' OH of existing nucleotide. Thus the addition of new nucleotide at 3' end make the growth of chain in 5'–3' direction. This 5'- 3' polymerization activity, is present in all DNA polymerase. DNA polymerase also has some other function like 5' – 3' exonuclease activity responsible for removal of nucleotide from polynucleotide strand and 3' – 5' exonuclease activity responsible for proofreading to check correct complementary nucleotide addition on a newly synthesized strand. All these three activities are not universal in all DNA polymerases. DNA polymerase also is known as "Replicas".

DNA polymerase in Prokaryotes

There is five DNA polymerase. Palm is conserved in all families but finger and thumb present as similar secondary structure elements from different sequences.

DNA polymerase I :

It was isolated by Arthur Kornberg in 1956 from E.Coli. It has single subunit and encoded by the polA gene. It is the first DNA polymerase to be identified. Polymerase I has three activities.

5'–3' exonuclease activity

3'-5' exonuclease and

5'–3' polymerase activities.

When Klenow treated the pol I with proteases. Pol I is cleaved by proteases such as subtilisin or trypsin it gives two fragments:

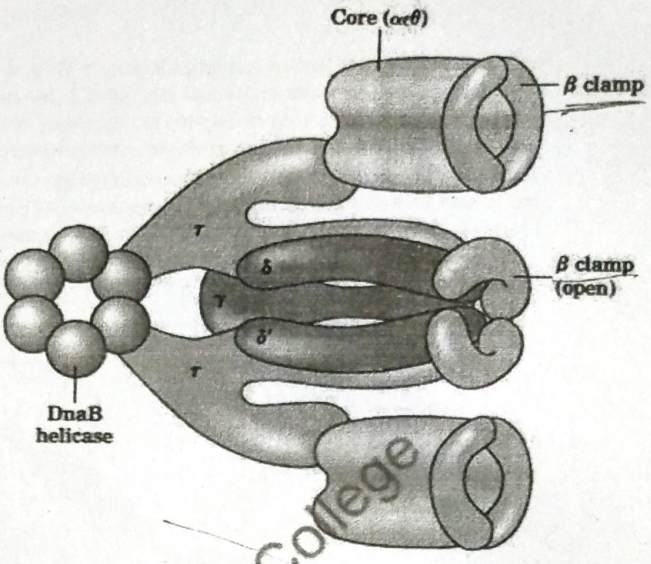
A larger C- terminal or Klenow fragment (residues 324–928 and mw 68 KD), has both the polymerase and the 3'-5' exonuclease activities.

N-terminal fragment (residues 1–323 and mw 35 KD) smaller one, has the 5'–3' exonuclease activity. Thus, Pol, I contain three active sites on a single polypeptide chain. It has 5 '-3' exonucleolytic activity coordinated with the synthetic/proofreading activity. Polymerization rate of Pol I is 10 to 20 nucleotides per second. Role of DNA polymerase in recombination, nick translation, excise RNA Primers and repair.

DNA polymerase III:

DNA polymerase III is a complex enzyme having ten types of subunits. Its polymerization and proofreading activities reside in its α and ε (epsilon) subunits, respectively. The θ subunit associates with α and ε to form a core polymerase, which can polymerize DNA but with limited processivity. Two core polymerases can be linked by another set of subunits, a clamp-loading complex, or γ complex, consisting of five subunits of four different types, $\tau_2\gamma\delta\delta'$. The core polymerases are linked through the τ (tau) subunits. Two additional subunits, χ (chi) and ψ (psi), are bound to the clamp-loading complex. The entire assembly of 13 protein subunits (nine different types) is called DNA polymerase III.

DNA polymerase III can polymerize DNA, but with a much lower processivity that will not be sufficient for replication of an entire chromosome. The necessary increase in processivity is provided by the addition of the β subunits The β sliding clamp prevents the dissociation of DNA polymerase III from DNA, dramatically increasing processivity—to greater than 500,000.



- DNA polymerase III is true replicase.
- In contains 10 types of subunits.
- Polymerisation activty by α subunit.
- Proof reading activity by ε (epsilon) subunit.
- Core polymerase will be —α,θ,ε'.

The gaps between fragments of the nascent lagging strand are filled by DNA polymerase I. This essential enzyme also uses its 59 n 39 exonuclease activity to remove the RNA primer lying ahead of the polymerase site. The primer cannot be erased by DNA polymerase III, because the enzyme lacks 59 n 39 editing capability. Finally, DNA ligase connects the fragments.

DNA polymerase II:

It has seven subunits and encoded by polB gene. DNA polymerase II play role in DNA repair when replication fork progress is blocked due to damage in DNA.

Primase

DNA polymerase can not initiate the DNA replication done. It require a 3'oH to add deoxyribonucleotide (dNTPs). That means to "prime" a reaction of polymerization of a monomer, the DNA polymerase synthesizes a short segment (sequence) of RNA that work as a primer. Primase is RNA polymerase. Thus DNA polymerase themselves cannot initiate DNA synthesis de novo. Primase is encoded by the dnaG gene in prokaryotes. In leading strand, only one primer requires but each Okazaki fragments has its own primer. In eukaryotes Pol α act as primase.

Helicases

Helicases are hexameric ring shape protein which is mechanically separate the two strands of double-stranded nucleic acid by translocation on one strand in ATP dependent manner. Helicase translocates on DNA strand in particular direction which is popularly known as a polarity of helicase means either move in 5' – 3'direction or 3'-5' direction. Helicase functions in a various event like DNA replication, recombination, repair, transcription termination, RNA splicing and RNA editing. A mechanism used by helicase to perform its function known as kinetic selectivity.

Single-strand binding protein

Helicase unwinds double-stranded DNA two single-stranded DNA and those single-stranded regions of DNA get prevented from annealing by binding of single-strand binding protein, (SSB) SSB because separated strand having tendency to get reassociate and form duplex thus allowing the DNA replication machinery to perform its function. SSBP present in viruses to humans. In Escheria coli SSB present in the homo tetrameric form.

In high salt concentration tetrameric form SSB65 binds to approximately 65 nucleotides of DNA, in contrast, low salt concentration dimeric form (SSB)35 binds to 35 nt DNA. The SSB binds in a cooperative manner means binding of one monomer facilitate the binding of the second monomer and further so no. In eukaryotes SSB present in a trimeric form known as heterotrimeric RPA (Replication Protein A). SSBs function as monomers in many phage and virus.

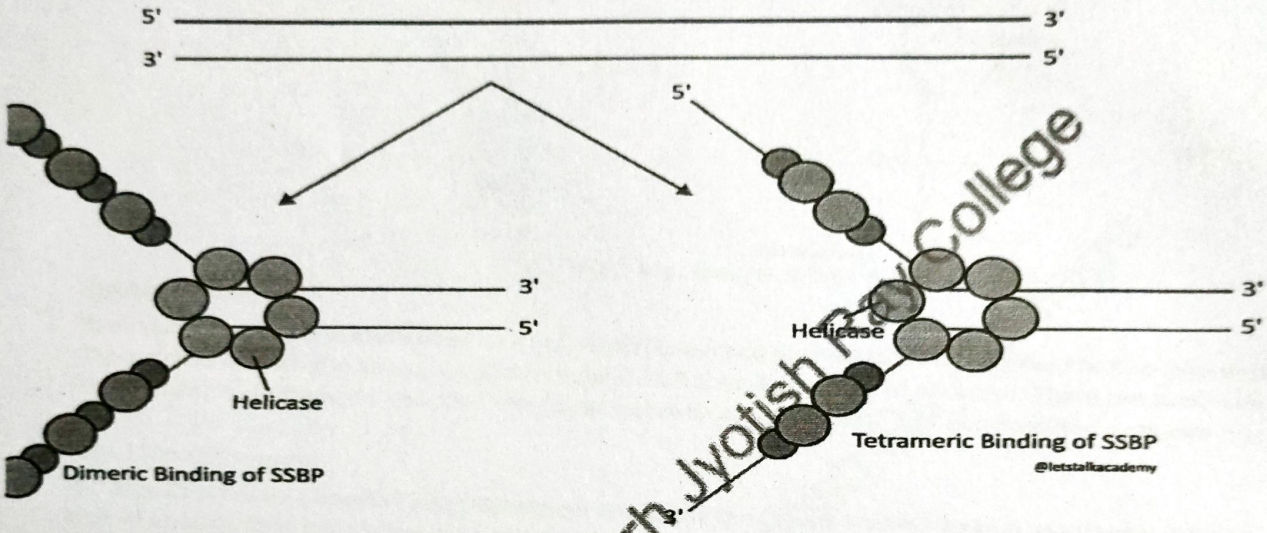


Fig. : Diagram showing binding of SSB protein to single stranded DNA to prevent re-annealing.

DNA ligase

Once the RNA primer has been removed and replaced by DNA through Pol I, then with the help of DNA ligase phosphodiester bond is formed between the adjacent Okazaki fragments One Okazaki fragment has 3' ọH end and another fragment of Okazaki has 5' phosphate end. These two ends are seal by DNA ligase. Nick remains in leading strand sealed by DNA ligase. DNA ligase also requires energy to make phosphodiester bond. NAD (nicotinamide adenine dinucleotide) work as a cofactor and source of energy for DNA ligase in E. coli whereas ATP used by T4 DNA ligase. DNA ligases are present in both prokaryotes and eukaryotes.

Both enzymes undertake a two-step reaction in first step DNA ligase interact with ATP and form an enzyme-AMP complex. The AMP in the enzyme-AMP complex becomes attached to the 5' phosphate of the nick, and then a phosphodiester bond is formed with the 3'-OH terminus of the nick, releasing the enzyme and the AMP.

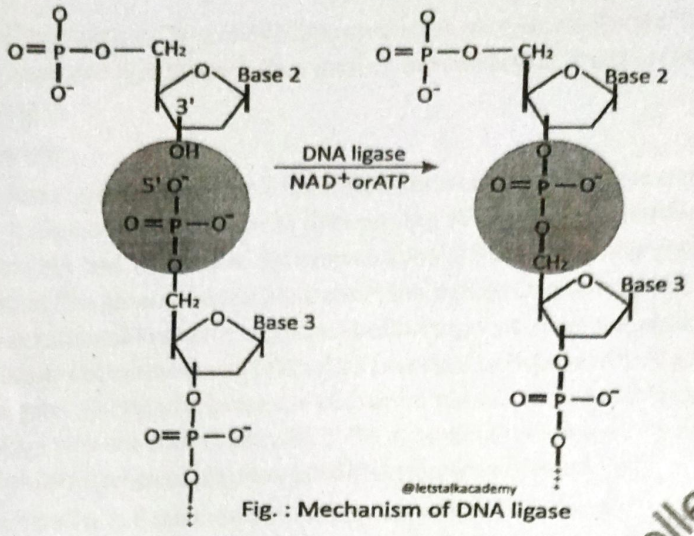


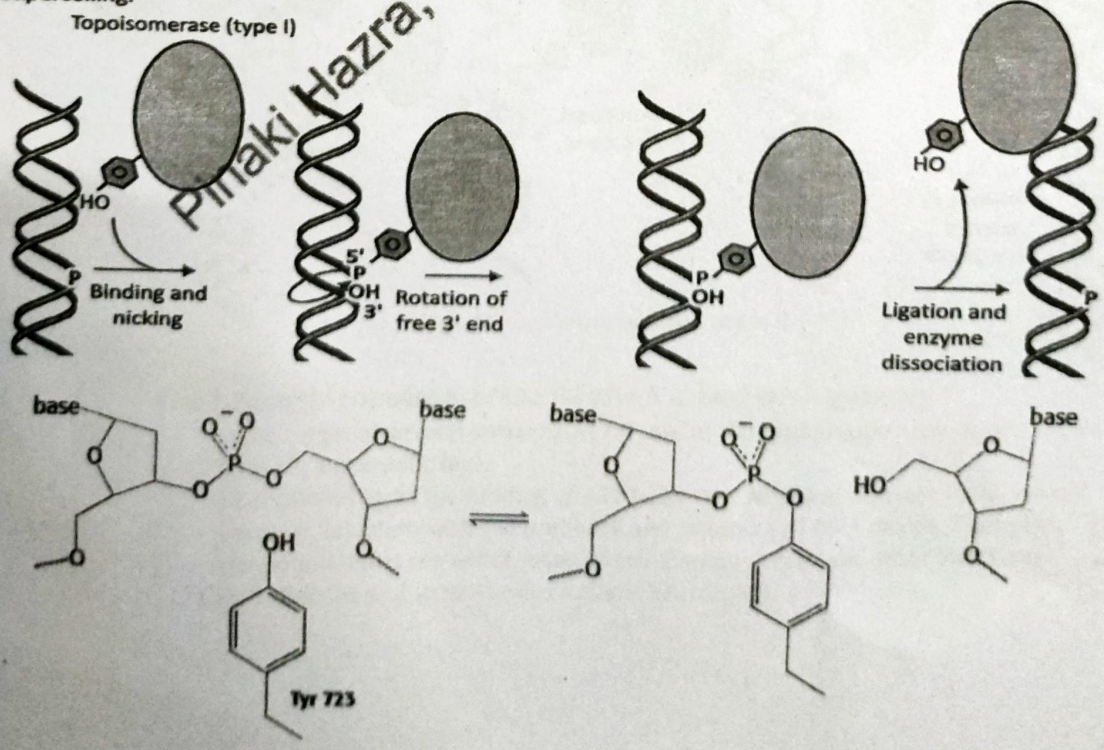
Fig. : Mechanism of DNA ligase

Topoisomerases

Topoisomerase is able to introduced nick in a single strand and double strand by cleaving the phosphodiester bond in single strand or both the strand of double-strand DNA thus it is a type of nuclease. There are two main classes of topoisomerase. In Escheria coli four individual topoisomerase present and get classified in above mention, two classes.

Type I Topoisomerase:

It is able to produce a break in one DNA strand and cause the linking number to increase by one. DNA binds with in cleft of enzyme thus get placed near active tyrosine. Active-site Tyr tyrosine attacks as a nucleophile and break a phosphodiester bond in one DNA strand, cleaving it and generating a covalent 5'-phosphotyrosyl protein- DNA linkage and free 3' OH cause the formation of open conformation of the enzyme, this result in a gap in cleaved strand and the gap bridge by enzyme. After that uncut strand passes through the gap cause the formation of the closed conformation of enzyme, as a result, liberated 3'-OH of cleave strand attacks the 5'-phosphotyrosyl protein-DNA linkage to religate the cleaved DNA strand. Type I Topoisomerase did not use ATP and responsible for catenane/decatenated, relaxation of supercoiling and knotting/ unknotting. E.g. Type I Topoisomerase which relaxes negative supercoiling in E.coli, Type III Topoisomerase of E.coli. and Type I Topoisomerase of calf thymus which will relax negative and positive supercoiling.



Type I divide into two subclasses; Type I which form covalent intermediate at 5' end of DNA with changing the linking number by one integer and Type IB which form covalent intermediate at 3' end of DNA with changing the linking number by two integers.

Type II Topoisomerase:

It is dimeric or in some cases tetrameric, able to produce a break in two DNA strand and cause the linking number to increase by two. It requires energy released by ATP hydrolysis to facilitate conformational change in enzyme not for cleave and rejoin DNA and responsible for catenation/decatenated, relaxation/induction of supercoiling and knotting/ unknotting. The general mechanism features the passage of one intact duplex DNA segment through a transient double-strand break in another segment. The DNA segment enters and leaves the topoisomerase through gated cavities above and below the bound DNA, which are called the N gate and the C gate, enter through N gate and release through C gate. Two tyrosine present in each active site subunit and each tyrosine form 5'-phosphotyrosyl protein- DNA linkage with one DNA strand with 3'-OH in other sites of cleave strand, two ATP require in over all process. The broken DNA is religated, and the second DNA segment is released.

E.g. Type II Topoisomerase in E.coli known as DNA gyrase which is a tetramer of two Gyr A subunit and two GyrB subunits, can introduce negative supercoils and decrease Lk (Linking Number) through the utilization of ATP. DNA strand cutting and rejoining done by GyrA which is inhibited by nalidixic a quinolone antibiotic and its fluorinated derivatives norfloxacin and ciprofloxacin. ATP hydrolysis did by GyrB which is inhibited by novobiocin. DNA gyrase uses in initiation of replication and transcription.

Other examples include Type IV Topoisomerase of E.coli. Type II topoisomerases of eukaryotes can relax both positive and negative supercoils but cannot unwind DNA or introduce negative supercoils in DNA. In vertebrates Type, II Topoisomerase has two isoform IIα and IIβ. In Archaea, DNA gyrase belongs to type IIA and topoisomerase VI belongs to IIB family of Type II Topoisomerase.

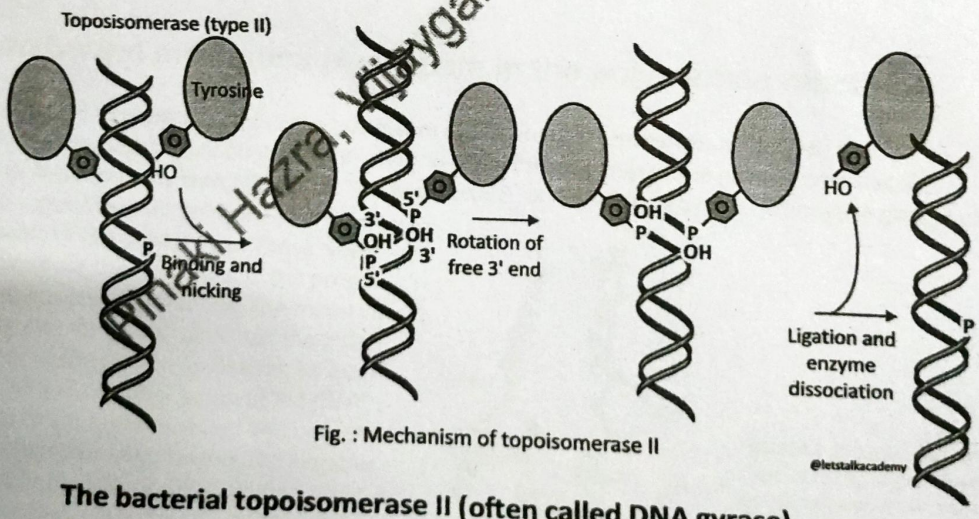


Fig. : Mechanism of topoisomerase II

The bacterial topoisomerase II (often called DNA gyrase) is the target of several antibiotics that inhibit the prokaryotic enzyme much more than the eukaryotic one.

Novobiocin blocks the binding of ATP to gyrase. *Nalidixic acid* and *ciprofloxacin*, in contrast, interfere with the breakage and rejoining of DNA chains. These two gyrase inhibitors are widely used to treat urinary-tract and other infections including those due to *Bacillus anthracis* (anthrax).

Camptothecin, an antitumor agent, inhibits human topoisomerase I by stabilizing the form of the enzyme covalently linked to DNA.

GYRASE

- An enzyme that changes the number of times the two strands in a closed DNA molecule cross each other It does this by cutting the DNA, passing DNA through the break, and resealing the DNA
- It's belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA

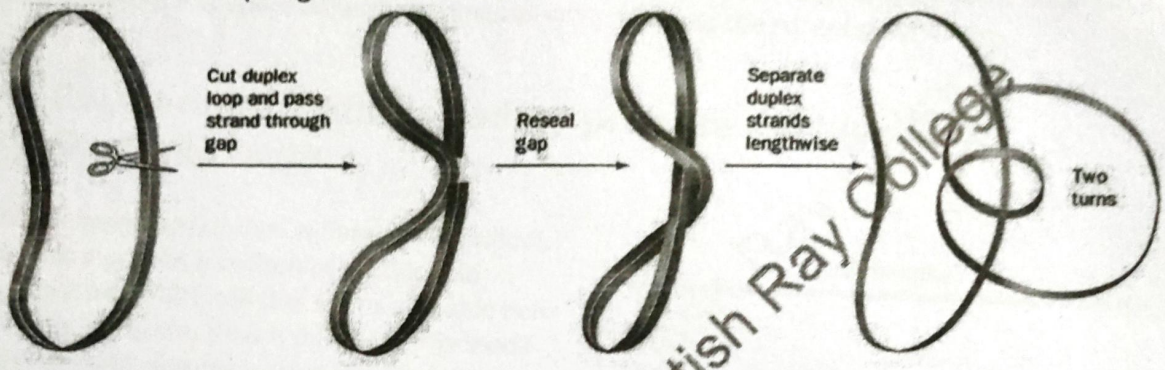


Figure 29-30 A demonstration that DNA gyrase acts by cutting both strands of a duplex, passing the duplex through the break, and resealing it.

Two bound metal ions participate in the polymerase reaction

Two metal ions are present in the active site of enzyme. One metal ion binds both the deoxynucleoside triphosphate (dNTP) and the 39-hydroxyl group of the primer, whereas the other interacts only with the dNTP . The two metal ions are bridged by the carboxylate groups of two aspartate residues in the palm domain of the polymerase. These side chains hold the metal ions in the proper positions and orientations. The metal ion bound to the primer activates the 3'OH group of the primer, facilitating its attack on the a phosphoryl group of the dNTP substrate in the active site. The two metal ions together help stabilize the negative charge that accumulates on the pentacoordinate transition state. The metal ion initially bound to Dntp stabilizes the negative charge on the pyrophosphate product.

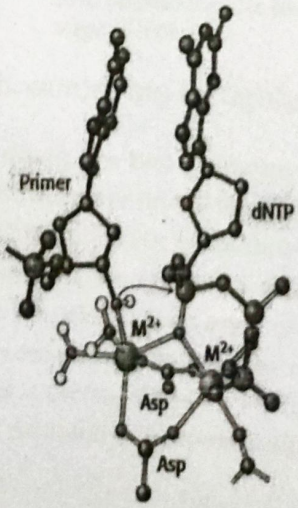


Figure 28.4 DNA polymerase mechanism. Two metal ions (typically, Mg^{2+}) participate in the DNA polymerase reaction. One metal ion coordinates the 3′-hydroxyl group of the primer, whereas the other metal ion interacts only with the dNTP. The phosphoryl group of the nucleoside triphosphate bridges between the two metal ions. The hydroxyl group of the primer attacks the phosphoryl group to form a new O–P bond.

An examination of the crystal structures of various DNA polymerases reveals why shape complementarity is so important.

First, residues of the enzyme form hydrogen bonds with the minor-groove side of the base pair in the active site In the minor groove, hydrogen-bond acceptors are present in the same positions for all Watson–Crick base pairs. These interactions act as a "ruler" that measures whether a properly spaced base pair has formed in the active site. Second, DNA polymerases close down around the incoming dNTP). The binding of a deoxyribonucleoside triphosphate into the active site of a DNA polymerase triggers a conformational change: the finger domain rotates to form a tight pocket into which only a properly shaped base pair will readily fit. Many of the residues lining this pocket are important to ensure the efficiency and fidelity of DNA synthesis. For example, mutation of a conserved tyrosine residue that forms part of the pocket results in a polymerase that is approximately 40 times as error prone as the parent polymerase.

An RNA primer synthesized by primase enables DNA synthesis to begin

DNA polymerases cannot initiate DNA synthesis without a primer, a section of nucleic acid having a free 3'OH end that forms a double helix with the template. How is this primer formed? An important clue came from the observation that RNA synthesis is essential for the initiation of DNA synthesis. In fact, *RNA primes the synthesis of DNA.* An RNA polymerase called *primase* synthesizes a short stretch of RNA (about five nucleotides) that is complementary to one of the template DNA strands .Primase, like other RNA polymerases, can initiate synthesis without a primer. After DNA synthesis has been initiated, the short stretch of RNA is removed by hydrolysis and replaced by DNA.

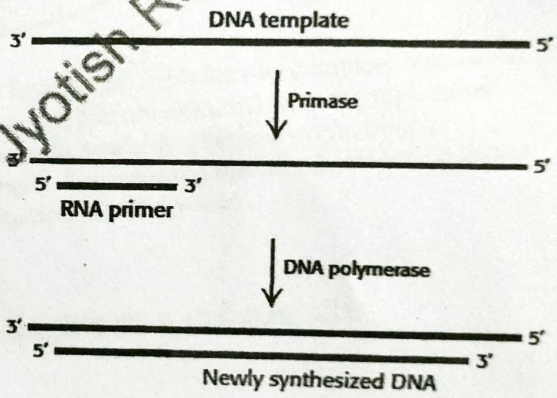
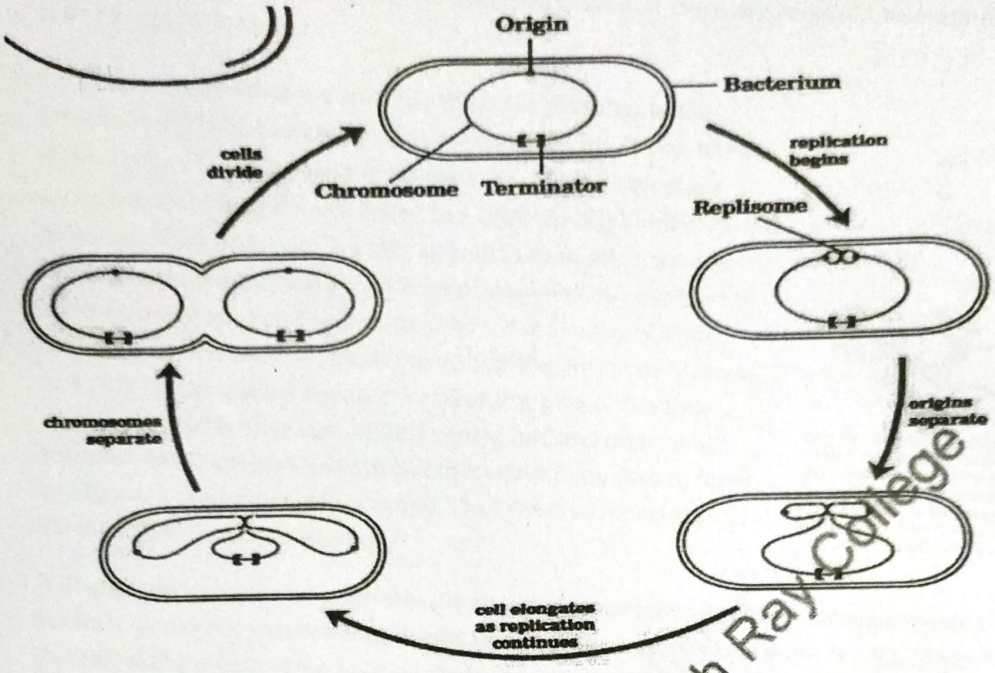


Figure 28.8 Priming. DNA replication is primed by a short stretch of RNA that is synthesized by primase, an RNA polymerase. The RNA primer is removed at a later stage of replication.

The replication of a circular bacterial chromosome is highly organized.

Once bidirectional replication is initiated at the origin, the two replisomes do not travel away from each other along the DNA. Instead, the replisomes are linked together and tethered to one point on the bacterial inner membrane, and the DNA substrate is fed through this —replication factory‖. The tethering point is at the center of the elongated bacterial cell. After initiation, each of the two newly synthesized replication origins is partitioned into one half of the cell, and continuing replication extrudes each new chromosome into that half. The elaborate spatial organization of the newly replicated chromosomes is orchestrated and maintained by many proteins, including bacterial homologs of the SMC proteins and topoisomerases.



All replication is carried out at a central replication factory that includes two complete replication forks. The two replicated copies of the bacterial chromosome are extruded from the replication factory into the two halves of the cell, possibly with each newly synthesized origin bound separately to different points on the plasma membrane. Sequestering the two chromosome copies in separate cell halves facilitates their proper segregation at cell division.

DNA replication in *Escherichia coli* begins at a unique site

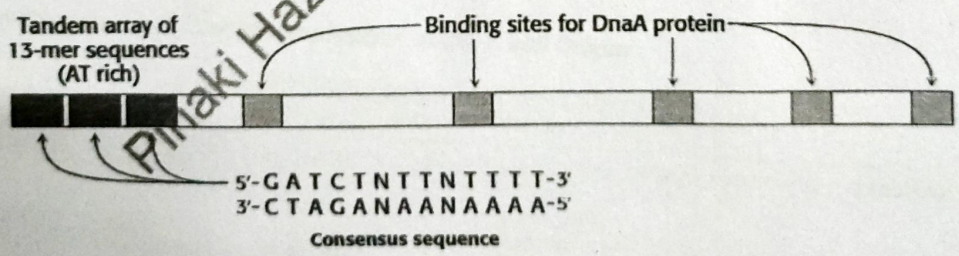
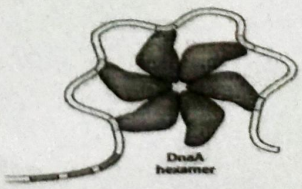


Figure 28.24 Origin of replication in *E. coli*. The *oriC* locus has a length of 245 bp. It contains a tandem array of three nearly identical 13-nucleotide sequences (green) and five binding sites (yellow) for the DnaA protein.

In *E. coli*, DNA replication starts at a unique site within the entire 4.6×10^6 bp genome. This *origin of replication*, called the oriC *locus*, is a 245- bp region that has several unusual features. The *oriC* locus contains five copies of a sequence that are preferred binding



sites for the origin-recognition protein DnaA. In addition, the locus contains a tandem array of 13-bp sequences that are rich in AT base pairs. Several steps are required to prepare for the start of replication:

1. ***The binding of DnaA proteins to DNA is the first step in the preparation for replication.*** DnaA is a member of the P-loop NTPase family related to the hexameric helicases. Each DnaA monomer comprises an ATPase domain linked to a DNA-binding domain at its C-terminus. DnaA molecules are able to bind to each other through their ATPase domains; a group of bound DnaA molecules will break apart on the binding and hydrolysis of ATP. The binding of DnaA molecules to one another signals the start of the preparatory phase, and their breaking apart signals the end of that phase. The DnaA proteins bind to the five high-affinity sites in *oriC* and then come together with DnaA molecules bound to lower-affinity sites to form an oligomer, possibly a cyclic hexamer. The DNA is wrapped around the outside of the DnaA hexamer

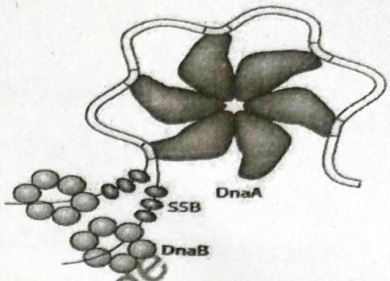


Figure 28.26 Prepriming complex. The AT-rich regions are unwound and trapped by the single-stranded-binding protein (SSB). The hexameric DNA helicase DnaB is loaded on each strand. At this stage, the complex is ready for the synthesis of the RNA primers and and assembly of the DNA polymerase III holoenzyme

2. ***Single DNA strands are exposed in the prepriming complex.*** With DNA wrapped around a DnaA hexamer, additional proteins are brought into play. The hexameric helicase DnaB is loaded around the DNA with the help of the helicase loader protein DnaC. Local regions of *oriC,* including the AT regions, are unwound and trapped by the single-stranded DNA–binding protein. The result of this process is the generation of a structure called the ***prepriming complex,*** which makes single-stranded DNA accessible to other proteins .Significantly, the primase, DnaG, is now able to insert the RNA primer.

3. ***The polymerase holoenzyme assembles.*** The DNA polymerase III holoenzyme assembles on the prepriming complex, initiated by interactions between DnaB and the sliding-clamp subunit of DNA polymerase III. These interactions also trigger ATP hydrolysis within the DnaA subunits, signaling the initiation of DNA replication. The breakup of the DnaA assembly prevents additional rounds of replication from beginning at the replication origin.

Proteins Required to Initiate Replication at the *E. coli* Origin:

Protein	*M,*	*Number of subunits*	*Function*
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*

Elongation stage of replication:

Elongation

In elongation, DNA polymerase polymerises the DNA strand by the addition of dNTP through the same mechanism.

In prokaryotes primer get removed by DNA polymerase I. DNA polymerase I bind at 3' OH of previous Okazaki fragment and cause the displacement of RNA base of a primer by it's 5'-3' exonuclease activity. Polymerase I also add new dNTPs at the 3' on end of Okazaki fragment by it's 5'-3' polymerisation activity. The last phosphodiester bond is bond by DNA ligase. In case of both leading and lagging strand and then synthesis of leading and lagging strand complete.

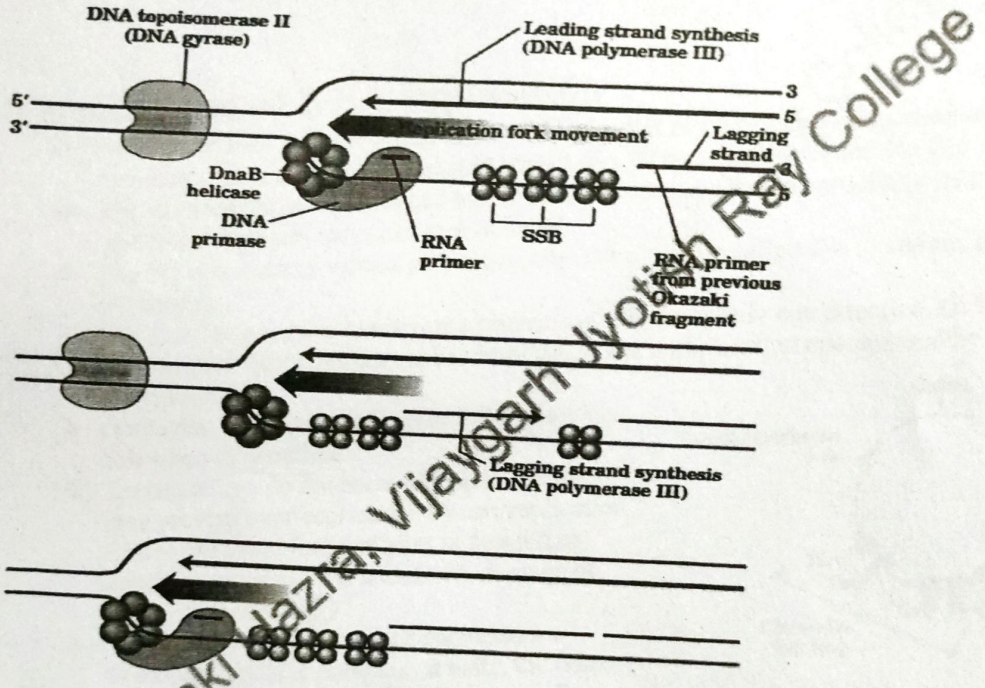


FIGURE: Synthesis of Okazaki fragments

- The entire complex responsible for coordinated DNA synthesis at a replication fork is a replisome.
- The replisome promotes rapid DNA synthesis, adding ~1,000 nucleotides/s to each strand (leading and lagging).
- Once an Okazaki fragment has been completed, its RNA primer is removed and **replaced with DNA by DNA polymerase I**, and the remaining nick is sealed by DNA ligase.
- DNA ligase catalyzes the formation of a phosphodiester bond between a 3' hydroxyl at the end of one DNA strand and a 5' phosphate at the end of another strand.
- The phosphate must be activated by adenylylation. DNA ligases isolated from viruses and eukaryotes use ATP for this purpose. DNA ligases from bacteria are unusual in that use NAD+.

Proteins at the *E. coli* Replication Fork:

Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	791,500	17	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps; excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

Termination of DNA replication

- Eventually, the two replication forks of the circular *E. coli* chromosome meet at a terminus region containing multiple copies of a 20 bp sequence called Ter (for *ter*minus).
- The Ter sequences are arranged on the chromosome to create a sort of trap that a replication fork can enter but cannot leave.
- The Ter sequences function as binding sites for a protein called Tus (*t*erminus *u*tilization *s*ubstance).
- The Tus-Ter complex can arrest a replication fork from only one direction. Only one Tus-Ter complex functions per replication cycle—the complex first encountered by either replication fork.
- Given that opposing replication forks generally halt when they collide.
- Ter sequences do not seem essential, but they may prevent over-replication by one replication fork in the event that the other is delayed or halted by an encounter with DNA damage or some other obstacle.
- When either replication fork encounters a functional Tus-Ter complex, it halts; the other fork halts when it meets the first (arrested) fork.
- The final few hundred base pairs of DNA between these large protein complexes are then replicated (by an as yet unknown mechanism), completing two topologically interlinked (catenated) circular chromosomes. DNA circles linked in this way are known as **catenanes**. Separation of the catenated circles in *E. coli* requires Topoisomerase IV (a type II topoisomerase). The separated chromosomes then segregate into daughter cells at cell division.

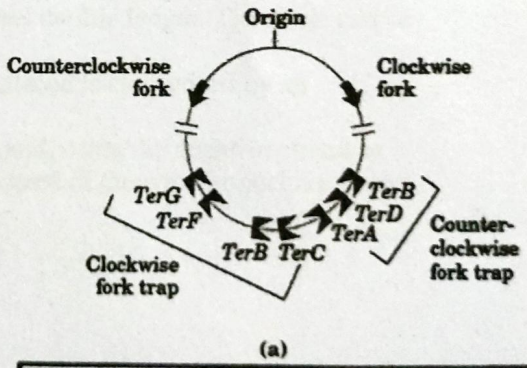


Fig: The Ter sequences are positioned on the chromosome in two clusters with opposite orientations

Special notes:

Unidirectional Replication:

Not all genetic systems follow bidirectional replication For example, replication of a plasmid called **colE1.** Replication of colE1 DNA is unidirectional.

Rolling Circle Replication:

Certain circular DNAs replicate, not by the common replication mechanism, but by a mechanism called **rolling circle** replication. For example, *E. coli* phages with single-stranded circular DNA genomes, such as □X174.

This mechanism the rolling circle name because the double-stranded part of the replicating DNA can be considered to be rolling counterclockwise and trailing out the progeny single-stranded DNA, rather like a roll of toilet paper unrolling as it speeds across the floor. This intermediate also somewhat resembles the lowercase Greek letter □□(sigma), so this mechanism is sometimes called the □ mode, to distinguish it from the θ□mode.

The rolling circle mechanism is not confined to production of single-stranded DNA. Some phages (e.g., □) use this mechanism to replicate double-stranded DNA.

Rolling circle DNA replication mechanism:

Schematic representation (given below) of rolling circle replication that produces single-stranded circular progeny DNAs.

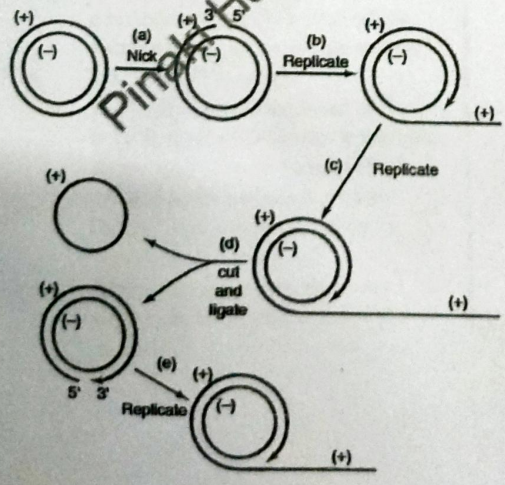
(a) An endonuclease creates a nick in the positive strand of the double-stranded replicative form.

(b) The free 3′-end created by the nick serves as the primer for positive strand elongation, as the other end of the positive strand is displaced. The negative strand is the template.

(c) Further replication occurs, as the positive strand approaches double length. The circle can be considered to be rolling counterclockwise.

(d) The unit length of positive-strand DNA that has been displaced is cleaved off by an endonuclease.

(e) Replication continues, producing another new positive strand, using the negative strand as template. This process repeats over and over to yield many copies of the circular positive strand.



Linear replication –

This the type of replication takes place in linear chromosome like in eukaryotes.

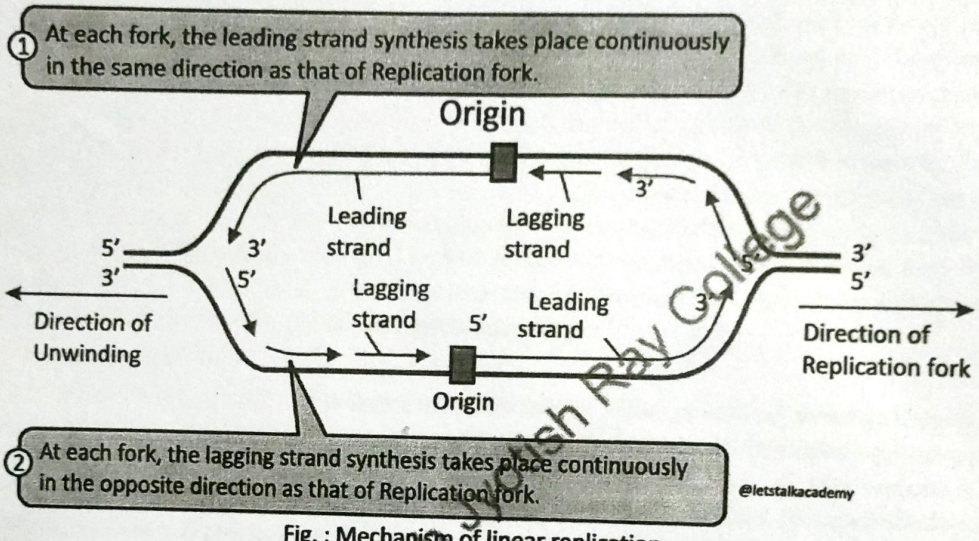
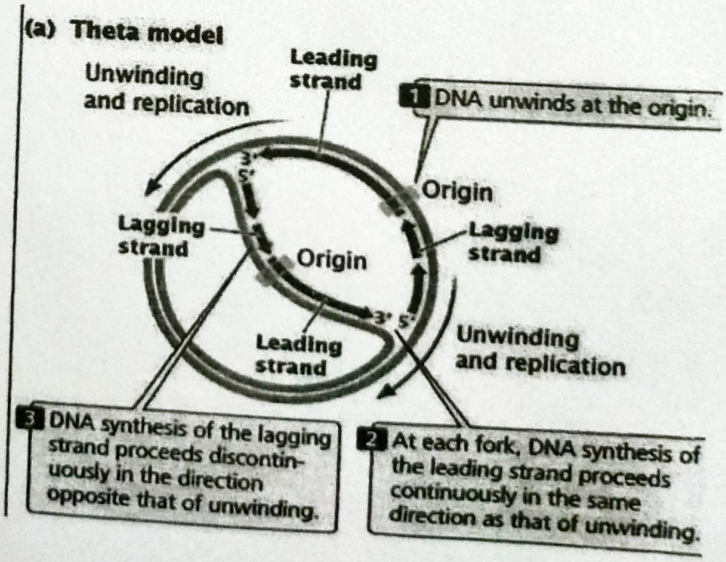


Fig. : Mechanism of linear replication

Theta (θ)mode of DNA replication

- During replication in bacteria(*E.coli*) a bubble is initiated at the origin and circular DNA molecule exhibits a θ (theta like)shape.
- Replication is bidirectional and both strands of DNA are replicated.
- As the two DNA strands in circular chromosome uncoils it creates coiling, which is removed by topoisomerase.
- As strands separates positive supercoils form else where in the molecule.



Replication of organelle DNA (Models for mtDNA replication)

DNA polymerase γ is used exclusively for mtDNA replication and proofreading. Two models have been proposed for the mode of mtDNA replication, 1) strand displacement model, appeal to continuous DNA replication and 2) coupled model, proposes semidiscontinous DNA replication.

Strand displacement model

The strand displacement model (also called the strand asynchronous model) for mammalian mtDNA replication is the most widely accepted, longest standing model. In strand displacement model, replication is unidirectional around the circle and there is one replication fork for each strand in which One strand is called the heavy strand (H) and another strand is called light strand (L). The designation of the strands as H and L due to their different buoyant densities in denaturing CsCl density gradients centrifugation. Two origins (OH and OL) because here one priming event per template strand and it starts within a region called the displacement or "D" loop of 500–600 nucleotides. The RNA primer synthesis by mitochondrial RNA polymerase. Firstly replication starts on H strand starting at OH. When DNA polymerase polymerise approximately two-thirds of the mtDNA as a result replication fork get passes the major origin of L strand synthesis, thus exposing the site in a single-stranded format which new H strand synthesis starts from OL. Synthesis is continuous around the circle on both strands. Multifunction endoribonuclease, RNase MRP cleave RNA primer. The same mechanism for cpDNA (cytoplasmic DNA) replication.

Strand coupled model

In strand coupled model at multiple sites lagging strand replication (L strand) get initiated, showing a discontinuous synthesis of short Okazaki fragments thus requiring multiple primers. So, in this model the coupled lagging strand and leading (H strand) synthesis represents a semidiscontinous, bidirectional mode of DNA replication. The endoribonuclease, RNase MRP (mitochondrial RNA processing) has at least two functions, 1) removal of RNA primers in mtDNA replication, 2) pre-ribosomal RNA's nucleolar processing. Mutation in RNase MRP cause cartilage-hair hypoplasia a rare form of dwarfism and due to its consequences multiple phenotypic manifestations (pleiotropy) occur which included short limbs, short stature, fine sparse hair, impaired cellular immunity, anaemia, and predisposition to several cancers.